Pacific Science (1997), vol. 51, no. 4: 462–474

A Hierarchical View of the Hawaiian Drosophilidae (Diptera)[1]

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ABSTRACT: As the pioneer natural historian of the Hawaiian entomofauna, R. C. L. Perkins showed a keen interest in the Diptera, in general, and the Drosophilidae, in particular. Perkins described and named two of the most charismatic of the Hawaiian picture-winged drosophilid flies: *Idiomyia heteroneura* and *I. silvestris.* These two species are part of a chromosomally homosequential quartet of species that have garnered the attention of research programs of numerous biologists. In this paper we review the evidence on the phylogenetic relationships among the flies in this quartet and suggest some guidelines for the inference of phylogeny within this quartet of species as further data accumulate. Perkins was also one of the first to recognize the extent of diversity of the Drosophilidae within and among islands of the archipelago. Several more-recent research programs have concentrated on understanding the evolutionary history of this diversification. Two questions regarding the high degree of diversity of these flies are discussed from a systematic perspective in this paper. The first concerns the relationships of the major species groups assemblages of the Hawaiian drosophilids. The second focuses on the origin of the Hawaiian drosophiloid and scaptomyzoid flies.

R. C. L. PERKINS RECOGNIZED that of all of the Diptera in Hawai'i, the Drosophilidae were unique in their degree of differentiation, speciation, and complex patterns of geographical distribution. Understanding this remarkable diversity and distribution has been a major research goal of drosophilists following Perkins' pioneering efforts. Over the past century, experimental approaches used to examine these flies have also been diverse, ranging from taxonomic studies to molecular biology. Phylogenetic relationships among the Drosophilidae in Hawai'i have been investigated at many hierarchical levels. A detailed understanding of the systematics of these flies can aid greatly in advancing our conceptions of their diversity and distribution and provide a framework for understanding the origin of evolutionary novelty from the molecular to the phenotypic level.

Much of the recent systematic work on the Hawaiian Drosophilidae has centered on higher-level relationships (Takada 1966, Throckmorton 1966, Kaneshiro 1976). In particular, systematists are interested in identifying the continental group that gave rise to the Hawaiian radiation and whether the two major groups of Drosophilidae found in the archipelago (drosophiloids and scaptomyzoids) arose as the result of one or two colonization events (Takada 1966, Throckmorton 1966, Stalker 1972). Systematic studies have also attempted to address the issue of the relationships of the various morphologically distinct species groups among the endemic drosophilids (Throckmorton 1966). In addition, the application of polytene chromosome analysis has advanced our understanding of species relationships within one of the species groups: the picture-winged *Drosophila* (Carson et al. 1967, Carson and Kaneshiro 1976). Shared patterns of banding reflecting historical inversions in polytene chromosomes were used extensively to examine the relationships of almost 100 picture-winged species. Allozyme studies, immunological studies, DNA-DNA hybridization, new morphological studies, and DNA sequence studies have subsequently been used to examine these older systematic questions as well as newer

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questions concerning biogeographic patterns of dispersal of these flies (DeSalle 1992, Kaneshiro et al. 1995). These techniques and their application are discussed in this paper to advance a more precise understanding of the relationships among these flies at several hierarchical levels of study.

The goal of this paper is to review recent DNA sequence and morphological studies and to assess their impact on our understanding of the systematics of Hawaiian drosophilids. In particular, we use recent molecular information, interpreted in a cladistic framework, to address three questions of systematic importance concerning these flies. First we examine the lower-level relationships of a now paradigmatic quartet of species, described by Perkins in 1910. Second, we examine the relationships of the various species groups composing the Hawaiian Drosophilidae implied by DNA characters and compare these with hypotheses obtained from morphology (Takada 1966, Throckmorton 1966, Kaneshiro 1976, Grimaldi 1990). We also examine whether these various species groups are monophyletic. Finally, we investigate the monophyly of the Hawaiian Drosophilidae (scaptomyzoid and drosophiloid relationships) and assess potential sister groups among continental drosophilid clades.

The planitibia *Species Quartet*

R. C. L. Perkins described two charismatic Hawaiian Drosophilidae, *Idiomyia heteroneura* and *I. silvestris,* which reside in the middle-elevation rain forests (900 to 1200 m) on the Big Island of Hawai'i. Later it was suggested that *Idiomyia* be synonymized with the genus *Drosophila* because of the close morphological affinity of flies in the two genera (Kaneshiro 1976; but see Grimaldi 1990). *Drosophila heteroneura* is striking because the male's head is extremely hypercephalic, perhaps a result of sexual selection. This pair of species has been the subject of intense behavioral, ecological, and genetical work (Carson 1970, Carson et al. 1970, Carson and Kaneshiro 1976, Kaneshiro 1983, DeSalle et al. 1986*a,b,* Kaneshiro and Boake 1987, Rowan and Hunt 1989). Two closely related, chromosomally homosequential species residing on geologically older islands in the archipelago (*D. planitibia* on Maui and *D. differens* on Moloka'i) are thought to be sister taxa to *D. heteroneura* and *D. silvestris.*

Although there is a great deal of intraspecific chromosomal inversion polymorphism within each species (see Carson 1982, 1990), there are no alternately fixed inversions among the four species. Isozyme studies likewise revealed high degrees of intraspecific variability, but again found no fixed differences among the four species (Johnson et al. 1975, Carson 1982). Morphological variation exists among the species, but it is, for the most part, not phylogenetically informative. For example, the extreme hypercephaly that is so prominent in *D. heteroneura* is unique to that species among the four. Variability in a foreleg bristle character has been the subject of several quantitative genetic studies, but this extra row of bristles appears only in some geographically distinct (Hilo side) populations of *D. silvestris* (which may represent a separate phylogenetic species). Other morphological characters, such as pigmentation on the wings and the faces of these flies, also appear to be diagnostic of individual species, but uninformative for relationships among species. Behavioral studies using the criterion of female choice as an indicator of phylogeny (the Kaneshiro hypothesis [Kaneshiro 1983]) suggest a close affinity of *D. heteroneura* with *D. differens.*

Although the morphological, chromosomal, and behavioral data have been the source of some interesting hypotheses concerning the relationships of this quartet of flies, no study to date has conclusively resolved the phylogenetic relationships of these flies. DNA sequence and restriction fragment length polymorphism (RFLP) data addressing this problem are discussed in detail here. Many of the hypotheses arising from alternate molecular studies are topologically incongruent. Therefore, we also discuss the interpretation of incongruence among these various sources of data. Before comparing these data and their congruence in detail, we outline our analytical methodology and review our philosophy of evidence in systematics.

Brower et al. (1996) argued that there are four kinds of incongruence that systematists must deal with in establishing systematic rela-

tionships. Type 1 incongruence is incongruence with nonempirical expectations, or informal notions of phylogeny. Such nonempirical expectations are viewed as conjectural starting points for more empirical approaches. Type 2 incongruence between trees results from attempting to compare patterns generated by different methods of analysis. When two data sets are analyzed by different approaches, such as phenetic versus cladistic methods, incongruence observed between the two phylogenetic hypotheses can arise as an artifact of the different methods, rather than as character incongruence intrinsic to the data. These first two types of incongruence therefore deal with cases where one or more topological hypotheses are not articulated by a cladistic analysis. The assessment of incongruence in these cases is impossible because of the incompatibility of data and data analysis, and hypotheses not supported by explicit character data should be ignored in favor of hypotheses that are so supported (see Brower et al. [1996] for a discussion). We emphasize that our decision to exclude certain kinds of data from the analysis does not indicate that these data are "worthless." Indeed any evidence that arises from empirical data collection is useful for establishing initial hypotheses. Our opinion that certain kinds of data should be excluded from this analysis simply indicates that these data are incompatible with our methodological approach. Their merits as sources of systematic evidence are addressed elsewhere (reviewed in Brower et al. [1996]). Type 3 incongruence is the situation where topological incongruence is claimed by a researcher based on visual inspection of competing hypotheses, but the incongruence is not supported by a rigorous analysis of the data included in the two competing hypotheses. DeSalle and Brower (in press) pointed out that Type 3 incongruence should now not be a problem because of the development of several statistical methods for the assessment of incongruence among data partitions (Farris et al. 1994, 1995, Larson 1994, Huelsenbeck et al. 1996). This leaves Type 4 incongruence, or competing phylogenetic signal among the different sources of evidence available for a phylogenetic study caused by character incompatibility between data partitions.

Analysis of separate gene partitions of the *Adh* gene region and the mitochondrial DNA RFLP characters results in the "discovery" of two strongly supported but discordant (incongruent) phylogenetic hypotheses for the DHPS quartet, which we shall call the "N" and "M" hypotheses, respectively (Figure 1*A,B*). To date, eight molecular data sets are available for these four species (mitochondrial DNA RFLP, and *Adh, Yp1, Ache, vg, CoII, wg,* and *hb* gene sequences [see caption of Figure 1 for full names of the genes]). Full cladistic analyses of these eight gene regions are given in DeSalle and Brower (in press). Two of the gene regions support the "M" topology (mtRFLP and *CoII*), three support the "N" topology (*Yp-1, Adh,* and *vg*), one is ambiguous in its support for these two hypotheses (*Ache*), and two are uninformative (*hb* and *wg*). The two types of congruence typically discussed in systematic literature (character and taxonomic congruence [Swofford 1991]) can be examined with these data.

Taxonomic (topological) congruence attempts to establish a consensus of taxonomic relationships from agreement among branching patterns implied by different data partitions. Putting our DHPS data in a taxonomic congruence framework results in the hypothesis in Figure 1*C*. All character sets are in agreement with a *D. heteroneura–D. silvestris* sister relationship. The disagreement among the partitions lies in the relationship of *D. planitibia* to this sister pair and to *D. differens*. Because the "M" phylogeny hypothesizes a pectinate relationship for *D. planitibia* and the "N" topology hypothesizes a sister relationship of *D. planitibia* with *D. differens,* the consensus of "M" and "N" results in *D. planitibia* and *D. differens* being unresolved. When the incongruence length difference (ILD [Mickevitch and Farris 1981]) is quantified and the statistical significance of this measure assessed (Farris et al. 1994, 1995), only two partitions are actually significantly incongruent: The mtDNA RFLP partition and the *Adh* partition show statistically significant (Farris et al. 1994, 1995) Type 4 incongruence; the other six genes do not contain sufficient phylogenetic information to disagree significantly with any other partition.

A simultaneous analysis of all the character information is also shown in Figure 1*D*. The cladogram is fully resolved and the "M" topology is preferred. Branch Bremer (1988, 1994)

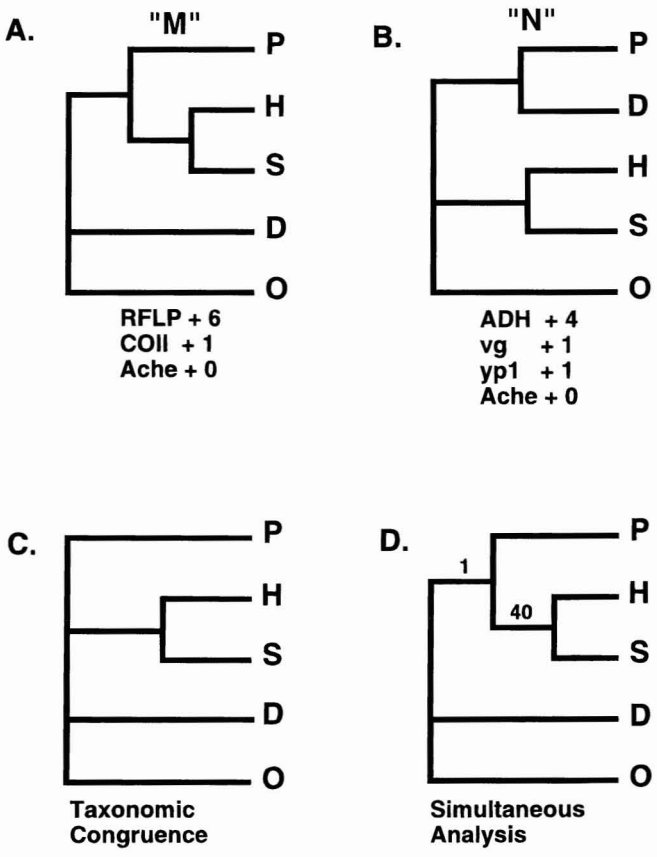

FIGURE 1. Results of a phylogenetic analysis of the individual partitions listed below for the *planitibia* species subgroup quartet: P = *planitibia,* H = *heteroneura,* S = *silvestris,* D = *differens,* O = outgroup (for details of sequencing and data analysis see DeSalle and Brower in press). *(A)* The "M" hypothesis. Phylogenetic analysis of the two mitochondrial (M) data partitions result in this topology. *(B)* The "N" hypothesis. Phylogenetic analysis of most nuclear gene partitions result in this topology. For *A* and *B* the numbers next to the genes below the cladograms refer to the decay index that the indicated partitions support the node defining the position of P. *Ache* was equivocal (+0) with respect to these two trees in that phylogenetic analysis resulted in both M and N as the most parsimonious solutions. *Hunchback* and *wingless* contained no phylogenetically informative sites and are not shown. *(C)* Taxonomic congruence and *(D)* Character congruence (simultaneous analysis) of multiple character sets listed below. The numbers at the nodes in the character congruence figure indicate the decay indices for the nodes. Partition abbreviations are as follows: *Ache,* Acetycholinesterase; *Adh,* Alcohol dehydrogenase; *COII,* Cytochrome oxidase II; RFLP, mitochondrial restriction fragment length polymorphisms; *yp1,* yolk protein 1; and *vg,* vestigial. Aligned sequences and the entire data set used to generate this figure are available on the AMNH Molecular Laboratories Web Page (http://research.amnh.org/molecular/index.html).

support for the *D. heteroneura–D. silvestris* sister-group arrangement is high (40), and the support for the node that establishes the critical difference between the "M" and "N" topology is low (1). In the absence of further information we accept the "M" topology as the best current representation of the phylogenetic hypothesis for these four taxa.

What is the "true" pattern of phylogeny in this group? We suspect that when more nuclear characters are collected they will eventually overwhelm the "M" topology and the "N" topology will thus be preferred. However, because of the equivocal nature of the three rather large nuclear character sets (*Ache, wg,* and *hb*) that we have most recently added to this analysis (DeSalle and Brower in press), it is not entirely certain that this will be true. Indeed, the nature

of systematics is unavoidably uncertain and hypothetical. Some may criticize our favoring the "M" topology as a way of ignoring the competing phylogenetic signal in the mtDNA RFLP partition and the *Adh* partition, but we point out that both the ILD analysis and a detailed examination of character change and homoplasy on the total evidence cladogram reveal the competing signal. Although we adopt a simultaneous analysis strategy to produce our phylogenetic hypothesis, we recommend strongly that tests for incongruence be performed. Our objection thus lies with the down-weighting of evidence based on subjective criteria to arrive at a phylogenetic conclusion different from that implied by parsimony.

The Relationship of Drosophilid Species Groups in Hawai'i

Although Perkins devoted much effort to describing the extensive morphological diversity among the Hawaiian *Drosophila,* he also recognized their evolutionary affinity and suggested that they were all members of the same taxonomic group. Later biologists such as Hardy (1965), Spieth (1966), Takada (1966), Throckmorton (1966), Carson (1970), and Kaneshiro (1976) corroborated Perkins' views and began the systematization of the group based mostly on morphological evidence. Hardy's (1965) impressive list of the species of Hawaiian *Drosophila* gave detailed descriptions of over 500 species of these flies in both the scaptomyzoid and drosophiloid groups. One thing that arose from this massive list of species was that the flies fell into several groups based on bizarre features of their external morphology. These species groups include the picture-winged, modified-tarsus, modified-mouthparts, white-tip scutellum, and antopocerus groups, and a few other smaller groups such as *Nudidrosophila* and *Atelidrosophila.* Table 1 shows the major species groups and the approximate number of species within each.

The relationships among the major species groups have been suggested based on the eggshell, genitalic, and internal morphology of these flies. Figure 2 summarizes some of these hypotheses. Both Takada (1966) and Throckmorton (1966) hypothesized a basal position for the white-tip scutellum group and derived positions for the picture-winged group and the modified-mouthparts group. Although Spieth (1966) did not construct a phylogenetic tree from his behavioral data, the data are amenable to cladistic analysis. Baker and DeSalle (in press) recoded the character state data from Spieth's (1966) Table 1 and obtained a phylogeny with the white-tip scutellum group of flies at the base of the tree (Figure 2*C*).

More recent molecular and morphological information has challenged the notions of monophyly of the various species groups. For instance, on the basis of distance information from the immunological precipitation technique, Beverley and Wilson (1985) suggested that the picture-winged group is not monophyletic. Specifically, they argued for the separation of the *adiastola* subgroup from the other picture-wings and suggested that this picture-winged subgroup is the most basal group of Hawaiian *Drosophila.* DeSalle et al. (1987) found corroborating support for these relationships based on the analysis of a single mitochondrial gene. Grimaldi's (1990) morphological revision of the family Drosophilidae used more complete taxon sampling than previous studies and included representatives of the five major morphological species groups, plus *Atelidrosophila* and *Nudidrosophila.* His cladogram (Grimaldi 1990: fig. 556), however, did not recover monophyly for any of the recognized species groups.

Recently, Baker and DeSalle (in press) used sequence data from eight gene regions (mt 16SrDNA, mt cytochrome oxidase III, mt cytochrome oxidase II, mt *ND-1,* nuclear alcohol dehydrogenase, nuclear acetylcholinesterase, nuclear *wingless,* and nuclear *hunchback*) from the flies listed in Table 1 as a source of character information for constructing a phylogenetic hypothesis. The morphological and behavioral data in Spieth (1966), Throckmorton (1966), and Grimaldi (1990) were not included in this analysis because of the lack of overlap of the taxa surveyed in the various studies. Baker and DeSalle (in press) used multiple representatives of each species group to test hypotheses of monophyly within species groups. At least two representatives of each of the five species groups were used in the analysis, and these were chosen from the different species subgroups within each

TABLE 1

THE FIVE SPECIES GROUPS OF HAWAIIAN *Drosophila* USED IN THIS STUDY

SPECIES GROUP	NO. OF SPECIES IN GROUP (approx.)	SPECIES USED IN THIS STUDY	ABBREVIATIONS
1. antopocerus	18	*D. adunca*	ANT
		D. tanythrix	
2. modified tarsus	150	*D. dasycnemia*	MTS
		D. petalopeza	
3. modified mouthparts	100	*D. mimica*	MMT
		D. soonae	
4. white-tip scutellum	100	*D. iki*	WTS
		D. sp.	
5. picture-winged	103		
planitibia subgroup	27	*D. silvestris*	PWP
		D. cyrtoloma	
adiastola	15	*D. adiastola*	PWA
grimshawi	65	*D. disjuncta*	PWG
		D. lineosetae	

species group. The molecular "total evidence" hypothesis in Figure 3 supports monophyly of all of the five major species groups. Support for these groups as indicated by high decay indices (range from 5 to 34) is quite strong in all cases except for the picture-winged group.

The relationships among the various species groups implied by the molecular data are also consistent with many of the previous hypotheses erected for the flies listed in Table 1. As in the phylogenetic trees generated from Spieth's (1966) data and Throckmorton's (1966) data, the DNA total evidence tree (Figure 3) suggests a basal position for the white-tip scutellum group of flies. The strong sister-group relationship of the antopocerus group and the modified-tarsus group (decay index of 11) on this tree is supported by Kaneshiro's (1976) analysis of genitalic morphology. Although he did not use a cladistic analysis of his morphological data, Kaneshiro nonetheless recognized an affinity of the genitalia of these two species groups. Carson (1970, 1987, Carson and Kaneshiro 1976, Carson and Yoon 1982) hypothesized the close affinity of the picture-wings and the modified-mouthparts flies on the basis of chromosomal inversion data, and this relationship is supported by the molecular data (Figure 3).

Establishment of a well-supported phylogeny for the Hawaiian *Drosophila* is critical to understanding both the pattern of morphological change within this diverse group and the molecular and developmental bases of these morphological transformations. For instance, the sister relationship of antopocerus flies and modified-tarsus flies implies that changes in the foreleg morphology are common to this lineage and suggests that foreleg development could be a fruitful subject of developmental research for these groups. Kambysellis et al. (1995) also commented on the importance of polarizing the ecological characters relevant to host shifts among these flies. The placement of the white-tip scutellum group of flies as the most basal Hawaiian drosophilid species group may have important implications for ecological research.

Endemism and Origin of the Hawaiian Drosophilidae

The issue of the origin of the Hawaiian Drosophilidae has been approached using several techniques. On the basis of morphological and ecological considerations, Throckmorton (1975) suggested that the ancestor of the Hawaiian Drosophilidae resided in Southeast Asia. Stalker (1972), using chromosomal inversion patterns, suggested that the *melanica-robusta* species group was most likely to be the closest continental drosophilid relative to the Hawaiian Drosophilidae. More recently, the cladistic morphological analysis of Grimaldi (1990) called into question many of these widely accepted notions of phylogeny and classification of these flies. His

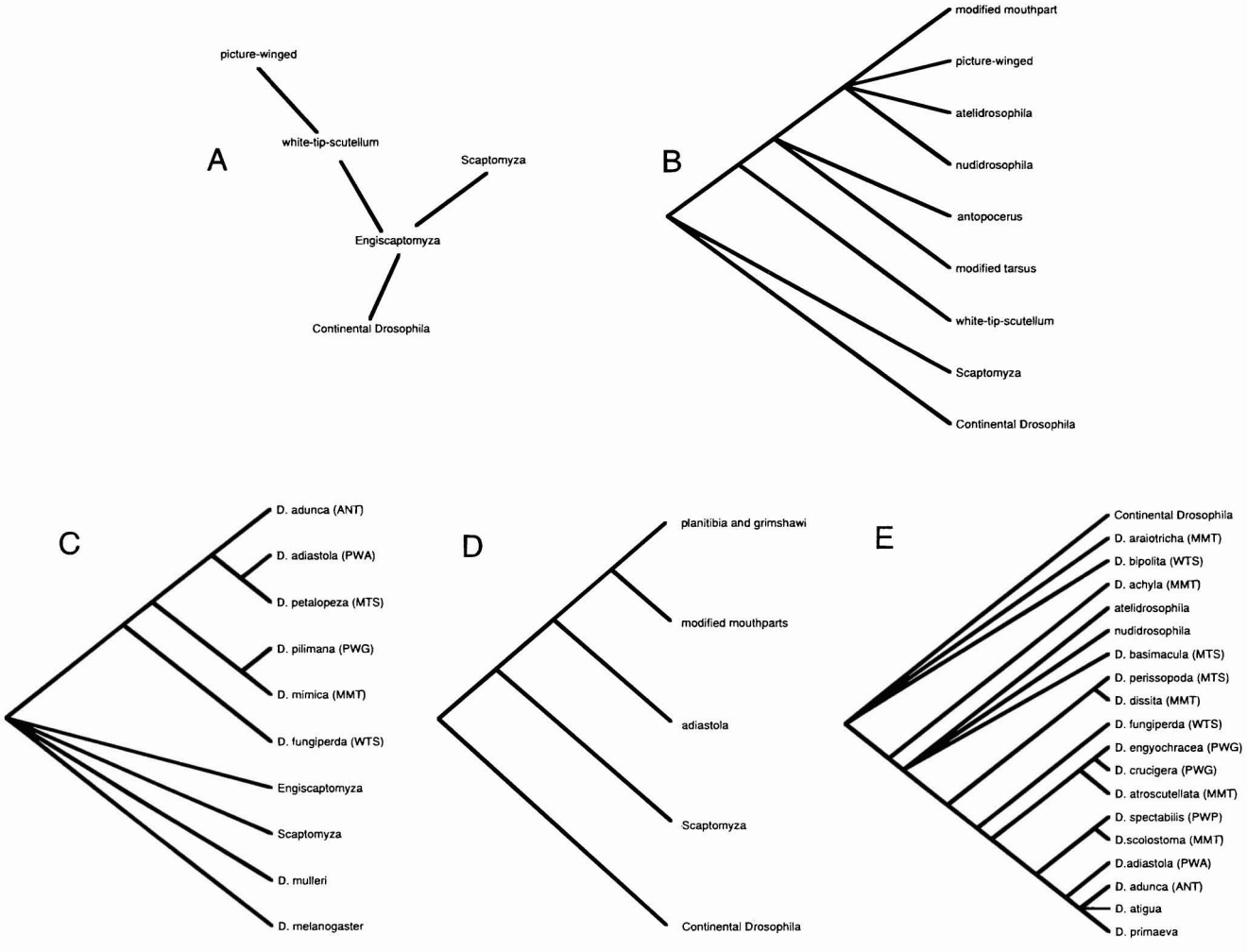

FIGURE 2. Five published hypotheses for the relationships of the various species groups of Hawaiian Drosophilidae. *(A)* The morphological hypothesis of Takada (1966) based on genitalia. *(B)* The morphological hypothesis of Throckmorton (1966) from his fig. 25. *(C)* A cladogram constructed from the behavioral data of Spieth (1966) using the character coded data from his Table 1. *(D)* Beverley and Wilson's (1985) UPGMA dendrogram concerning

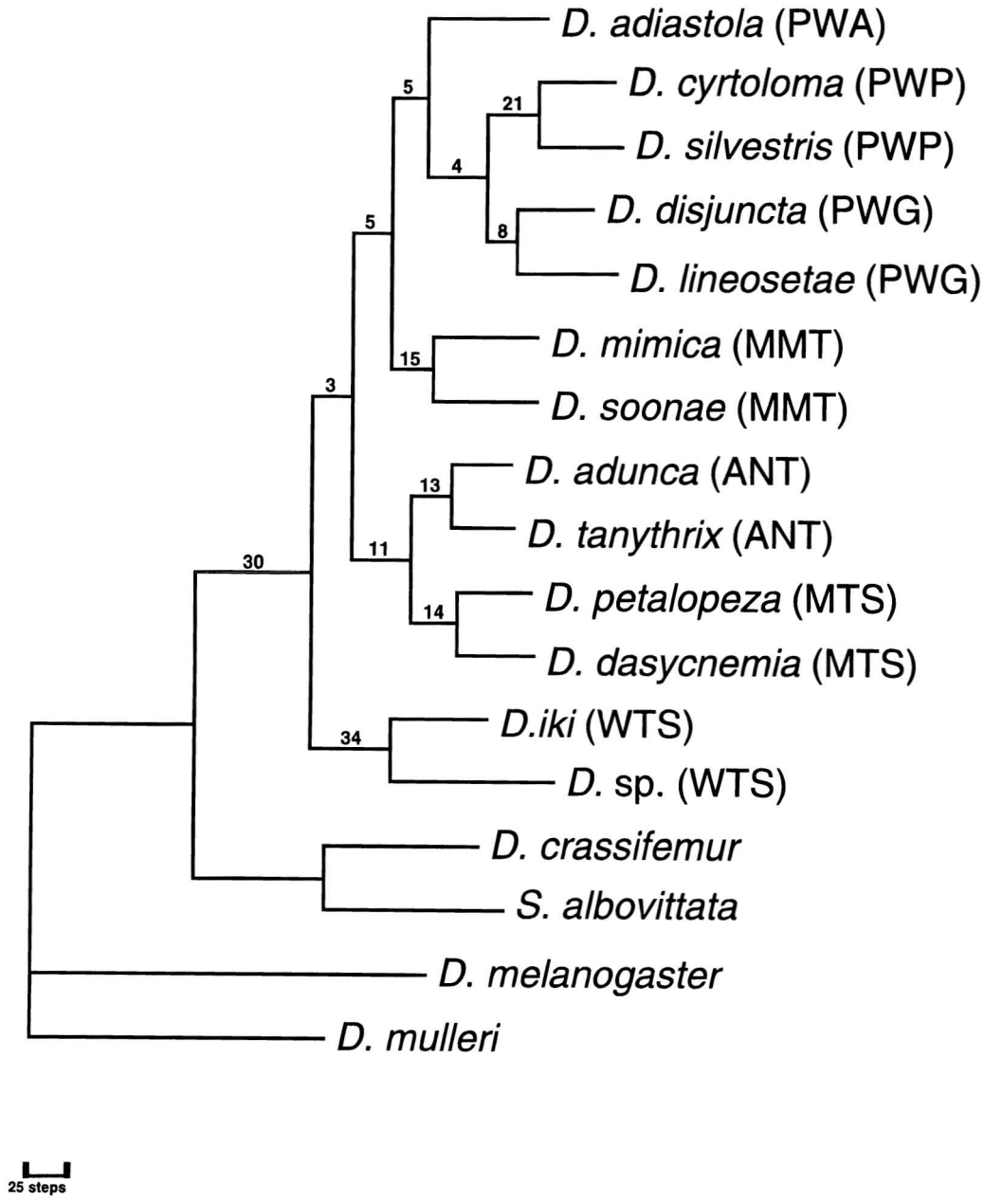

FIGURE 3. The simultaneous analysis hypothesis for the Hawaiian drosophilid species groups depicted as a phylogram where branch lengths reflect the amount of apomorphic change (see scale bar). Abbreviations for the species-group names are given in Table 1. Numbers above the nodes are branch support values. Outgroups are *Scaptomyza albovittata, Drosophila crassifemur, D. melanogaster,* and *D. mulleri.* The entire data set used to generate this figure is available on the AMNH Molecular Laboratories Web Page (http://research.amnh.org/molecular/index.html).

analysis questioned two of the more widely accepted notions of monophyly in the group.

First, Grimaldi (1990) suggested that the scaptomyzoid flies found in Hawai'i are not the sister group to the drosophiloid flies. Rather, his data suggested that they are monophyletic but related to other genera in the family Drosophilidae. This is in direct contrast to Throckmorton's (1966) contention that the scaptomyzoids and drosophiloids could indeed be monophyletic and could have arisen from a single introduction to the Hawaiian Islands. However, Throckmorton (1966) also considered the possibility of two introductions of the scaptomyzoids and drosophiloids, and that scenario would not necessarily require the two groups to be monophyletic. DNA sequence data, on the other hand, suggest a close relationship of the scaptomyzoid lineage with the drosophiloid lineage (DeSalle 1992, Thomas and Hunt 1993).

The second unconventional result hypothesized by Grimaldi (1990) suggests that a mycophagous clade of drosophilids including *Hirtodrosophila* and *Zygothrica* are the sister group to all the drosophilids found in Hawai'i. This suggestion hypothesizes a basal and very distinct position with respect to the genus *Drosophila* for these flies. However, in molecular studies using the *Adh* gene (Thomas and Hunt 1993) and more complete taxonomic sampling using mitochondrial sequences (DeSalle 1992), as well as in combined molecular-morphological studies (DeSalle and Grimaldi 1991, DeSalle 1993), the Hawaiian species are observed as sister to the subgenus *Drosophila.* The subgenus *Drosophila*–Hawaiian Drosophilidae clade was observed as the sister to the subgenus *Sophophora,* meaning that the Hawaiian taxa are embedded in the genus *Drosophila.* One potential problem with the molecular analyses is the sparse taxon sampling. In typical molecular studies to date, the relationship of the Hawaiian flies to other flies in the family is assessed by including at most six potential sister taxa. Grimaldi's (1990) study included over 60 genera and 160 taxa.

Remsen and DeSalle (in press) have used a simultaneous analysis approach to examine this question of the origin of the Hawaiian taxa. Their taxonomic sampling scheme is not too different from that in DeSalle (1992), but they included both the morphological data from Grimaldi (1990) and Throckmorton (1966) for these taxa as well as the following DNA sequence characters: the superoxide dismutase gene (Kwiatowski et al. 1994; with new information for several continental species, continental genera, and Hawaiian taxa added); the 28S nuclear rDNA gene (Pelandakis and Solignac 1993; with new information for several continental species, continental genera, and Hawaiian taxa added); the *Adh* gene (Thomas and Hunt 1993; with new information for several taxa added); and mitochondrial DNA (DeSalle 1992). The simultaneous analysis topology derived from these data is shown in Figure 4. Two long-standing questions pertinent to the Hawaiian Drosophilidae can be addressed with this cladogram: the origin of the group and the monophyletic status of the scaptomyzoid and drosophiloid flies.

On the basis of mitochondrial DNA sequences, DeSalle (1992) suggested that the Hawaiian taxa were the sister group to all other taxa in the subgenus *Drosophila* and that no single continental group could be posited as the sister to the Hawaiian taxa. The important phylogenetic result with respect to the origin of the Hawaiian taxa in Figure 4 (Remsen and DeSalle, in press) is that the Hawaiian Drosophilidae appear to be in a clade containing the *repleta-virilis* pair and the *melanica-robusta* pair. This recalls Stalker's (1972) original suggestion that members of the *melanica-robusta* species group were the closest relatives of the Hawaiian taxa on the basis of chromosomal inversions. Support for the nodes pertinent to this question is fairly robust (note the corresponding Bremer indices), although there exists a discrepancy between our result (Figure 4) and previously reported hypotheses concerning this phylogenetic question (DeSalle 1992, Powell and DeSalle 1995). More molecular characters and greater taxon sampling should add a greater degree of resolution to the question. The cladogram in Figure 4 also supports a sister-group relationship for the Hawaiian scaptomyzoid and drosophiloid lineages. This arrangement confirms Throckmorton's (1966) contention that a single introduction initiated the radiation of flies in the family Drosophilidae in the Hawaiian archipelago.

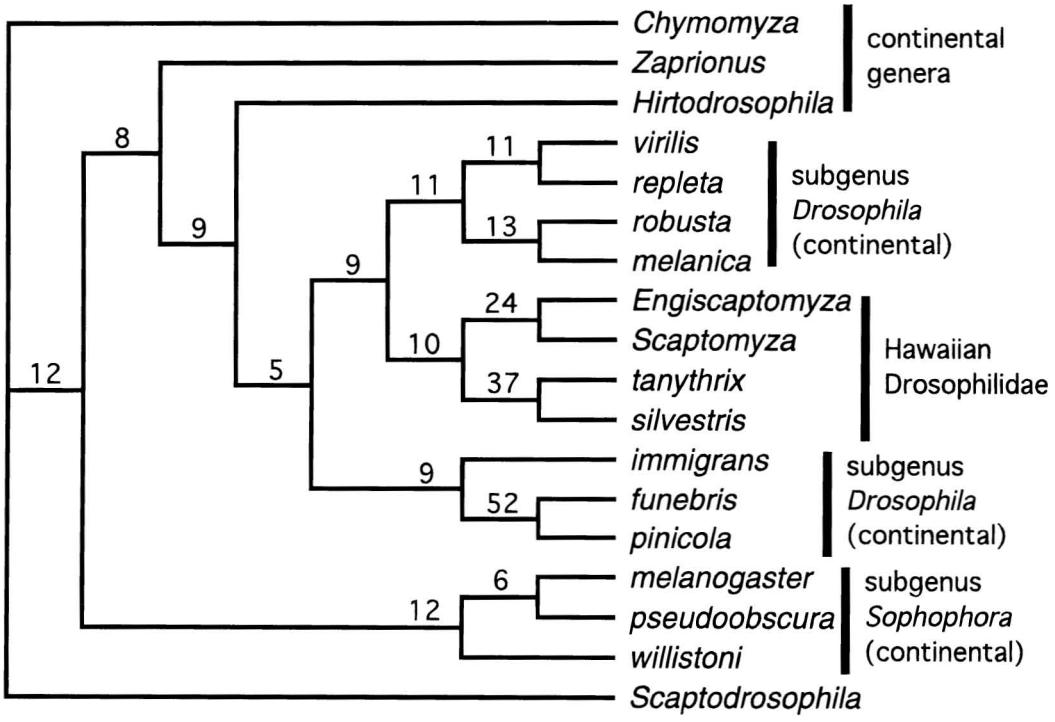

FIGURE 4. A phylogenetic hypothesis (Remsen and DeSalle, in press) based on information from 16S mitochondrial DNA, *Adh,* Superoxide dismutase (SOD), 28S nuclear rDNA, and morphology. Representatives of several species groups as well as several genera are included in the cladogram. Numbers at the nodes refer to branch support and are included as an indicator of robustness of support for particular nodes. The entire data set used to generate this figure is available on the AMNH Molecular Laboratories Web Page (http://research.amnh.org/molecular/index.html).

Conclusion

We have touched upon three phylogenetic questions concerning the Hawaiian Drosophilidae using cladistic approaches. Each question focuses on a different hierarchical level of the phylogenetic history of these flies using simultaneous analysis of multiple character partitions to infer phylogenetic hypotheses. In some cases this approach yields robust hypotheses. For instance, the relationships of the various species groups of Hawaiian drosophilids are strongly supported at almost all nodes (Figure 3). In other cases, such as the placement of *D. planitibia* (Figure 1), the inference is weak, despite the availability of a large number of phylogenetically informative molecular characters. Measures of character congruence, such as the ILD (Mickevich and Farris 1981), and a detailed understanding of character change at these three hierarchical levels can guide our understanding of competing phylogenetic signal among different sources of characters and choice of new sources of molecular characters. Scrutiny of behavior and utility of molecular characters in this way will be very important if we are to approach the more interesting and intriguing phylogenetic questions in this group of flies.

Furthermore, phylogenetic hypotheses of relationship will become increasingly important tools to build a better understanding of the adaptive radiation that the Hawaiian Drosophilidae have undergone at all hierarchical levels. Patterns of relationship among closely related species will be most important in corroborating biological scenarios for the colonization of the Hawaiian archipelago by these flies. The lack of strong resolution we observed in the DHPS quartet (Figure 1) suggests that some of the key relationships may be difficult to infer with confidence, because the apparent rapid radiation of

flies in the quartet is documented only by a few phylogenetically informative characters. Some higher-level relationships, such as the branching order of species groups deep in the phylogeny of Figure 3, may also be difficult to resolve because phylogenetic radiation may entail weak phylogenetic signal.

Cladistic relationships will also provide the comparative framework for the examination of phylogenetic trends in morphology, ecology, and development. Without hypotheses of relationship for these flies, conjectures concerning the transformation of ecological preference or developmental programs remain essentially untestable. It is therefore important that rigorous and robust phylogenetic hypotheses be developed across the range of taxonomic levels. Because the species-group level appears to be the most important for morphological (and to a lesser extent ecological) diversification, we suggest that the results summarized in Figure 3 are an important step toward this end. Finally, establishment of the sister group to the Hawaiian taxa in conjunction with fossil data (Grimaldi 1987, DeSalle 1992, Powell and DeSalle 1995) will allow for a detailed and more precise temporal calibration of the radiation of these flies. The tempo and mode of phylogenetic and ecological radiations can only be approached through a precise understanding of the sister-group relationships of these flies (Figure 4).

Perkins' interest in the Hawaiian entomofauna in general and the Diptera in particular opened the way for the Hawaiian Drosophilidae to become one of the premier paradigms in evolutionary biology. His early documentation of these unique flies and his recognition of the extent of their diversity were the first steps in understanding the unique biology of these flies. Our hierarchical phylogenetic perspective represents only one of the many facets of Perkins' biological legacy that is still being fulfilled.

ACKNOWLEDGMENTS

We thank James Liebherr and Dan Polhemus for organizing the Legacy of R. C. L. Perkins Symposium at the International Congress of Entomology in Firenze, Italy, and for the invitation to present the work summarized in this paper. Thanks are also given to two reviewers for comments on the manuscript.

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